Tropical Medicine and International Health doi:10.1111/tmi.12418

VOLUME 20 NO 2 PP 205–210 FEBRUARY 2015

Preliminary survey of domestic animal visceral leishmaniasis and risk factors in north-west Ethiopia

Ambaye Kenubih[1], Shimelis Dagnachew[2], Gizat Almaw[3], Tamerat Abebe[4], Yegnasew Takele[5], Asrat Hailu[4] and Wessensegad Lemma[6]

1 *Department of Veterinary Microbiology, Immunology and Public Health, Addis Ababa University, Debre Ziet, Ethiopia*
2 *Department of Para-Clinical Studies, Faculty of Veterinary Medicine, Gondar University, Gondar, Ethiopia*
3 *National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia*
4 *Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*
5 *Faculty of Medicine, Gondar University, Gondar, Ethiopia*
6 *Leishmania Research and Training Center, Gondar, Ethiopia*

Abstract

OBJECTIVE After the epidemics of *L. donovani* complex in 2004/05 in human patients, to investigate the presence of antibodies against *L. donovani* in domestic animals in north-west Ethiopia.
METHODS Two hundred and three domestic animals were screened. Serum and biopsy samples were collected. A modified direct agglutination test (DAT) for canine reservoirs was used to screen serum samples at ≥ 1:320 cut-off titre. Giemsa stain and culture on Novy macNeal Nicolae (NNN) media were used for biopsy samples. Pre-tested questionnaires were used to elicit information on potential risk factors.
RESULTS Antibody against *L. donovani* in domestic animals was detected in 30.5% of animals. The highest seropositivity rates were 41.9% in cattle, 40% in dogs, 33.3% in donkeys, 10% in goats and 4.8% in sheep. No *Leishmania* parasite was isolated from spleen, liver, skin snip and exudates, bone marrow or lymph node of dogs. Dogs owned by households with history of kala-azar treatment and humans sharing the house with cattle were more affected by visceral leishmaniasis ($P < 0.05$).
CONCLUSION This study showed a high serological prevalence of leishmaniasis in domestic animals. Their role in the epidemiology of visceral leishmaniasis remains unclear.

keywords *L. donovani*, antileishmanial antibodies, kala-azar treatment, risk factor, domestic animal, Libo Kemkem, north-west Ethiopia

Introduction

Leishmaniasis is caused by a diphasic protozoan parasite of the genus *Leishmania*. In East Africa, visceral leishmaniasis (VL) is endemic in parts of Sudan, Ethiopia, Somalia and Kenya. It causes at least 4000 deaths annually and a loss of approximately 385,000 disease-adjusted life years (DALY) (Reithinger *et al.*, 2007). The overlapping geographical distribution of VL and AIDS in this region significantly increased mortality rates (Horst *et al.* 2008) and (Alvar et al. 2008) has reported has the highest co-infection rate reported in the world.

In Ethiopia, long-recognised VL-endemic foci lie in the lowlands (<1500 metre above sea level); these are situated in Metema and Humera in the north-west, bordering east Sudan (Mengesha & Abuhay 1978). This region accounts for approximately 60% of VL cases in the country and reflects the first ecologic pattern. Other ecological patterns prevail in the regions of Lake Abaya, Omo River and the lower Omo, Segen and Woyto valleys in the south and south-west (Fuller *et al.* 1979; Ayele & Ali 1984; Ali & Ashford 1994). Sporadic epidemiological surveys showed the presence of VL in more than 40 isolated localities in Ethiopia. A recent outbreak of VL in the highlands of NW Ethiopia, Libo district, south Gondar claimed hundreds of lives (Alvar *et al.* 2007) before medical interventions were put in place.

In Sudan, Ethiopia, Kenya and Somalia, *L. donovani sensu lato* is the cause of visceral leishmaniasis (VL) and anthroponotic transmission seems to predominate (Soulsby 1982). Post kala-azar dermal leishmaniasis (PKDL) patients also play an important role in VL transmission (Sundar & Rai 2002). Some wild animals are incriminated as a reservoir host in Sudan, such as the grass rat (*Arvicanthis niloticus*, *Rodentia*: *Muridae*), the spinus mouse (*Acomys albigena*, *Rodentia*: *Muridae*), the serval (*Felis serval*, *Carnivora*: *Felidae*) and the genet (*Genitta genitta*, *Carnivora*: *Viverridae*) (Hoogstraal &

Heyeneman 1969), the jackal (*Canis* spp) (Sixl *et al.* 1987) and the Egyptian mongoose (Elnaiem *et al.* 2000). Natural leishmaniasis by *L. infantum* is reported in horses (Ramos-Vara *et al.* 1996), cats (Sollano–Gallego *et al.* 2000) and goats (Williams *et al.* 1991), but their role in vector infectivity has not been studied. Dogs are incriminated as a reservoir host in east Sudan for *L. donovani* (Dereure *et al.* 2000, 2003) and in Mediterranean region for *L. infantum* (El Harith *et al.* 1989).

In the Horn of Africa, there are two distinct ecologic settings of visceral leishmaniasis: the semi-arid regions where *Phlebotomus orientalis* breeds in cracks in the black cotton clay soil (Hoogstraal & Heyeneman 1969) and the savannah and forest areas in the south, where the vectors *P. martini* and *P. celiae* are found in association with *Macrotermes* termite mounds (Gebre-Michael & Lane 1996).

The main vectors of VL in Ethiopia are *P. martini* and *P. celiae* in south (Gebre-Michael & Lane 1996) and *P. orientalis* in the south-west (Hailu *et al.* 1995). Studies implicate *P. orientalis* as the most probable vector of VL in Humera and Metema endemic areas of NW Ethiopia, and it is highly suspected in the recent outbreak areas of Libo district (Gebre-Michael *et al.* 2010) as well as in Bellessa high land valley in the north (Ashford *et al.* 1973).

The purpose of this study was to detect the presence of antibody for visceral leishmaniasis in selected domestic animals and to identify potential risk factors in the cycle of the disease

Materials and methods

Study area

Libo Kemkem wereda is located in the Amhara Regional state, north-western Ethiopia, at an altitude of 2,000 metres above sea level. The district is made up of 30 *kebeles* (administrative units) with an estimated population of 196,813 in 2004. Addis Zemen (the district capital, population of 19,755) is located between Bahir Dar and Gondar on the major road connecting Addis Ababa to dry port Galabat, Sudan which crosses known foci of intense VL transmission in Metema, Ethiopia and Gedaref, East Sudan. The district has one health centre and 10 health posts.

It was in two *kebeles* (Bura sand Egziarab) of Libo Kemkem wereda where the first VL outbreak of 2004 occurred and claimed hundreds of lives. These villages were selected purposively to detect the rate of infection and antibody development in domestic animals. Amhara Regional State reported the outbreak as Quartum-resistant malaria, but a later epidemiological investigation by Médécain Sans Frontieres Greece (MSF-G) proved that it was VL with up to 7% cumulative incidence (Alvar *et al.* 2007). The village comprises 1200 households. Agriculture is mainly rain-fed, and very little irrigation is practiced in the winter season.

Study design

This was a cross-sectional study based on a convenience sample of domestic animals: dogs (n = 90), cats (n = 3), cattle (n = 43), donkeys (n = 15), sheep (n = 42) and goats (n = 10). This study was approved by the Ethical Committee of Animal Welfare of the Research and Publication Office of Gondar University.

Sample collection

Serum samples were collected aseptically from the jugular vein of cattle, sheep, donkey and goats or from cephalic veins of dogs and cats and were allowed to clot at room temperature. Serum was separated by centrifuging at 1500g for 2 min and collected into a sterile vial and stored at −20 °C.

Biopsies were taken after obtaining informed consent from the owners, and fine needle percutaneous aspiration was employed to take spleen, lymph node, liver and bone marrow aspirates from seven dogs. These were immediately smeared on a sterile glass slide and fixed with methanol for Giemsa stain and cultured in Novy MacNeal Nicolae (NNN) media in the field and later incubated in the laboratory to isolate the parasite. Standard surgical procedures and biopsy materials were applied to get fine needle aspirates from ingunal lymph node, liver, spleen and bone marrow aspiration from trocantric fosa of the femur bone by applying 2% lignocaine hydrochloride local infiltration. Serological tests were carried out in the Leishmania research and training center, Gondar.

Diagnostic methods

We used the direct agglutination test (DAT) and parasitology (Giemsa stain and culture methods) to diagnose *Leishmania* in domestic animals (dogs, cattle, donkeys, goats and sheep). DAT is a highly sensitive (92–100%) and specific (72–100%) test to monitor low levels of antibody in canine reservoirs for *Leishmania donovani* (Mohebali *et al.* 2006). DAT was performed according to the modified method of El Harith *et al.* (1989) for canine and human reservoirs; a 1:320 titre was taken as the cut-off. The test was conducted using V-shaped microtitre plates. Both negative control (from diluent and antigen)

and positive control (from confirmed human VL patent) were applied. Serum samples were diluted serially at 1:20 to 1:20480 by transferring 50 μl of diluted serum and discarding the same amount from the last dilution. The diluent used was a solution containing 0.15 M NaCl, 0.2 M 2-Mercaptoethanol and 1% (vol/vol) foetal calf serum (SIGMA). After serial dilution, 50 μl DAT antigen (KIT, Amsterdam) were added to the sera in a microwell plate containing 50 μl of diluted serum, and the plates were shaken clockwise and anticlockwise to prevent splashing. After 18 h of incubation at room temperature, the DAT reading was taken over white board. The agglutination activity was detected visually and by comparing with the negative and positive human sera used as a control. In order to detect the possible cross reaction between *Trypanosoma spp* and *Leishmania spp*, animals were screened for trypanosome.

Parasitology

Smears prepared from biopsy of spleen, lymph node, skin snip and bone marrow were fixed with absolute methanol, stained with Giemsa and examined carefully by light microscope at high magnification (1000 × oil immersion) for the presence of Donovan bodies(amastigote stages).

Biopsies of ingunal lymph node, liver, skin snip and exudates, bone marrow and spleen of dog were cultured on Novy macNeal Nicolae (NNN) media, then incubated at room temperature and examined weekly over a 6-week period at different times. Growth of the parasite was detected by using inverted microscope and Giemsa staining. NNN media was prepared from nutrient agar containing 10% whole rabbit blood overlaid with liver infusion tryptose broth containing 200,000 IU/ml penicillin G and 1 μg/ml strepotomycine.

Data collection and analysis

Data were collected using pre-tested questionnaires to evaluate the involvement of potential risk factors, such as a history of kala-azar in the owners (treated for the disease or not), living near the dump and sharing the house with domestic animals at night. Dogs were physically examined at the time of taking biopsy aspirates.

The data were analysed by logistic regression with Intercooled Stata version 11. A statistically significant association between variables was said to exist if the computed *P*-value was below 0.05.

Results

The overall proportion of domestic animals with *Leishmania* antibody in DAT was 30.54% (62 of 203 animals (Table 1). The highest seropositivities of 41.9% and 40 were found in cattle and dogs, respectively. 92 sera collected from dogs had an agglutination reaction; 25.6% had a titration of 1:320 and 15.6% had a titration >320. Sera collected from cattle showed a high rate of agglutination reaction activity against *L. donovani* coomassie blue-stained antigen: of the 43 tested sera, 41.9% had ≥320. Sera collected from donkeys had a medium rate of agglutination activity ($n = 15$); 33.3% had a titre ≥1:320. Sera collected from sheep showed a very low agglutination activity ($n = 42$); 4.7% had a titre of ≥1:320. No parasite was observed from cultured samples and stained slides.

Dogs sharing the house with owners who had been treated for kala-azar had a high proportion of DAT positivity and high risk of infection (OR 8.9375, $P = 0.016$) (Table 3); sharing the house with the owners also showed a high risk of exposure (OR 5.464, $P < 0.001$) (Table 3).

Cattle and dogs sharing the house with owners who had been treated for kala-azar had a high risk of exposure to the parasite (OR 4.667, $P < 0.001$), but there is no relationship between cattle owned by treated and non-treated owners (OR 0.8839, $P = 0.927$) (Table 3).

Sheep showed a weak relation among owners who had or had not been treated for kala-azar and shared the house or had a separate house (OR 0.625 $P = 0.945$ and OR 1.2777, $P = 0.725$) (Table 3).

Twenty six tissue aspirates (Lymph node, liver, skin snip, spleen and bone marrow) from six dogs with positive DAT titres were examined for the presence of amastigotes by culture in NNN media. None was positive.

Table 1 Proportion of DAT-positive animals against *L. donovani* domestic animals using direct agglutination test (DAT)

Description	Dogs	Cats	Cattle	Donkey	Sheep	Goats	Total
No. animals examined	90	3	43	15	42	10	203
No. of positive(≥1:320)	36	0	18	5	2	1	62
Proportion (%)	40	0	41.86	33.3	4.8	10	30.5

Dogs owned by Kala-azar-treated owners were seropositive in a very high proportion than their counter parts (Table 2), and most of dogs are clinically normal but serologically positive ($P < 0.01$).

Discussion

In 2004, there was an active transmission of VL in the study area (Bura and Egziarab) and up to 7% cumulative incidence of human cases and two dog cases (of 40 examined) (5%) was reported due to *L. donovani* complex (Alvar *et al.* 2007). In our study, November 2008 to April 2009, a higher occurrence was observed in dogs, where 40% (36/90) were DAT-positive. This difference could be a result of the time gap between the human outbreak and canine outbreak. The high prevalence seen in our data may reflect our sampling after the epidemic (2004/5). In Brazil, high canine seroprevalence preceded the human epidemic by several years (Werneck *et al.* 2007).

A study in Sudan (Mukhtar *et al.* 2000) using DAT showed 68.7% (66/96), 21.4% (9/42) and 8.5% (5/59) in donkeys, cows and goats, respectively, using DAT for human patients with cut-off value ≥1:3200 (El *et al.* 1988). Six dogs and 25 sheep sera were also tested; none were reactive. Compared to our result of 33.3% (5/15), 41.9% (18/43) and 10(1/10) in donkeys, cattle and goats, respectively, the figures reported by Mukhtar *et al.* (2000) were high only in donkeys but lower in cattle and goats. The authors used the old version of DAT to screen human VL patients, whereas we used the modified version of DAT to detect asymptomatic carrier in canine and vulpine reservoirs (El Harith *et al.* 1989).

In an endemic village in Eastern Sudan, 43–74% of the dogs were seropositive by indirect fluorescent antibody test (IFAT) (Dereure *et al.* 2003). In Eastern Sudan, impression smears made from lymph node aspirations of 33 dogs were culture negative, but using *Leishmania*-specific PCR, 87 dog blood samples from the study area were positive, and there is a high correlation between DAT and PCR (Hassan *et al.* 2009). Negative parasitological results do not rule out *Leishmania* infection in dogs (El Harith *et al.* 1989). Domestic dogs may act as infection reservoirs, but large outbreaks are usually thought to involve anthroponotic transmission (Dereure *et al.* 2003).

Screened animals were negative for *Tryponosoma spp*, which possibly interfere with the DAT test. Cutaneous leishmaniasis (CL) was not diagnosed in the study area during the outbreak (Alvar *et al.* 2007).

In our study, the highest seropositivity of 41.9% was found in cattle while other similar study Bhattarai *et al.* (2010) in Nepal found goats to have high seropositivity 16% (23/144) followed by cattle 5% (1/20) and buffalo 4% (1/24). A cattle study in Bangladesh detected prevalences of 9.4% (n = 138) using ELISA and 3% (n = 138) using DAT. But the parasite DNA is not detected using Ln PCR and loop-mediated isothermal amplification (LAMP) (Alam *et al.* 2011). However, the absence of *Leishmania* DNA in cattle indicates clearly that cattle do not play a role as reservoir host, although these animals play an important role in the epidemiology of the disease. ELISA-based blood meal analysis of 273 fresh-fed *P. orientalis* females collected from Metema revealed a remarkably high bovine blood feeds (92%) with only 2.2% of human blood feeds (Gebre-Michael *et al.* 2010). Thus, they contract the parasite and develop detectable antibody against the parasite, but the mechanisms of parasite survival and inducing disease in cattle are unknown. Cattle may provide zooprophylaxis and the application of

Table 2 Proportion of animals owned by owners treated for Kala-azar and not treated for Kala-azar

	Dog	Cattle	Sheep
KRx			
(>320)	22	9	1
(<320)	16	16	18
Total	28	25	19
Proportion	57.89%	36%	5.26%
KN			
(>320)	8	7	1
(<320)	44	11	22
Total	52	18	23
Proportion	15.38%	38.89%	4.35%
CI	3.713–12.304	0.462–8.053	0.3975–0.9660
P value	0.016	0.927	0.725

KRx mean owners treated for Kala-azar.
KN means owners not treated for Kala-azar.

Table 3 Logistic regression models of risk factors for visceral leishmaniasis among domestic animals in Libo Kemkem, Ethiopia

Species	Risk factors	Odds ratio	Standard error	*P*-value	95% CI
Dog	Sharing the same house	5.464	0.4937	<0.001	2.0478–14.58
	Kala-azar treatment history	8.9375	0.5021	0.016	3.713–12.304
Cattle	Sharing the same house	4.6667	0.8591	<0.001	0.5059–11.018
	Kala-azar treatment history	0.8839	0.6382	0.927	0.462–8.053
Sheep	Sharing the same house	0.625	1.452	0.945	−0.5437 to 0.280
	Kala-azar treatment history	1.2777	1.4488	0.725	0.3975–0.9660

insecticide to cattle could cause sand flies to increase their feeding on humans, where the principal vector is *P. orientalis* (Kolaczinski *et al.* 2008).

On the Indian subcontinent, *P. argentipes* is the principal vector, which is 5 times more attracted to cattle than humans. Much of the blood meal of sandflies comes from cattle or goats (Bern *et al.* 2010). Cattle sharing the stable have a strong correlation of seroconversion (OR 4.6667, $P < 0.001$) (Table 3). Sandflies are rarely attracted to sheep due to their hairy nature.

Dogs owned by households with a history of treated kala-azar and sharing the house with humans are more affected by VL (OR 8.9375, $P = 0.016$) (Table 3). Khanal *et al.* (2010) showed that the presence of serologically positive goats increases the risk of being DAT positive in humans. Even if goats are not a reservoir, they play a role in the distribution of *L. donovani,* particularly in new VL foci. In Kenya, an autochthonous leishmaniasis case affecting domestic goat was found (Williams *et al.* 1991).

Sand flies may feed on the blood from other mammals including domestic animals such as equines (WHO 1991). In our study, 33.3% DAT-positive result shows that donkeys are exposed to the parasite. An IFAT-based study in equines detected 40% positives (Villalobos *et al.* 2010).

In our household survey, we also detected two PKDL and one ocular (uvitiis) form of post kala-azar leishmaniasis cases, similar to findings of Alvar *et al.* (2007) who recorded 3 PKDL cases. In Sudan, 50% of treated VL cases develop PKDL (Zijilstra *et al.* 1995). Due to budget and logistic limitations, we were unable to examine wild and intradomicillary mammals or perform molecular examination of DAT-positive animals and entomological studies.

Acknowledgements

This study was funded by a Gondar University Research and Publication Office (RPO) Grant. Laboratory reagents were supplied by the Leishmania Research and Training Center, Gondar, Ethiopia. Neither had a role in study design, data collection and analysis, decision to publish or preparation of the manuscript for publication.

References

Alam MS, Ghosh D, Khan GM *et al.* (2011) Survey of domestic cattle for anti-Leishmania antibodies and Leishmania DNA in a visceral leishmaniasis endemic area of Bangladesh. *BMC Veterinary Research* **7**, 1746–6148.

Ali A & Ashford RW (1994) Visceral leishmaniasis in Ethiopia. IV. Prevalence, incidence and relation of infection to disease in an endemic area. *Annals of Tropical Medicine and Parasitology* **88**, 289–293.

Alvar J, Bashaye S, Daneil A *et al.* (2007) Kala azar out break in libo kemkem, Ethiopia: epiderological and parasitological assessment. *American Journal Tropical Medicine Hygiene*, **77**, 275–282.

Alvar J, Aparicio P, Abrham A *et al.* (2008) The relationship between leishmaniasis and AIDS: the second 10 years. *Clinical Microboil Reviews* **21**, 334–359.

Ashford RW, Hutchinson MP & Bray RS (1973) Kala-azar in Ethiopia: epidemiological studies in a highland valley. *Ethiopian Medical Journal* **11**, 259–264.

Ayele T & Ali A (1984) The distribution of visceral leishmaniasis in Ethiopia. *American Journal of Tropical Medicine and Hygiene* **33**, 568–601.

Bern C, Courtenay O & Alvar J (2010) Of Cattle, Sand flies and men: a systematic review of risk factor analyses for South Asian visceral leishmaniasis and implications for elimination. *PLoS Neglected Tropical Diseases* **4**, e599.

Bhattarai NR, Auwera GV, Rijal S *et al.* (2010) Domestic animals and epidemiology of visceral leishmaniasis, Nepal. *Emerging Infectious Diseases* **16**, 231–237.

Dereure J, Boni M, Pratlong F *et al.* (2000) Visceral leishmaniasis in eastern Sudan: a short report. *Trans Roy* **94**, 154–155.

Dereure J, El safi SH, Buchiton B *et al.* (2003) Visceral leishmaniasis in eastern Sudan: parasite identification in human and dog; host parasite relationship. *Microbes and Infection* **5**, 1103–1108.

El Harith A, Slappendel RJ, Reiter I *et al.* (1989) Application of direct agglutination for detection of specific anti-leishmania antibodies in the canine reservoir. *Journal of Clinical Microbiology* **27**, 2252–2257.

El Harith AE, Kolk AH, Leewenburg J *et al.* (1988) Improvement of a direct agglutination test for field studies of visceral leishmaniasis. *Journal of Clinical Microbiology* **26**, 1321–1325.

Elnaiem DA, Hassan MM, Maingon R *et al.* (2000) The Egyptian mongoose, *herpestes ichreumon*, is a possible reservoir host for VL in eastern Sudan. *Parasitology* **122**, 531–536.

Fuller GK, Lemma A, Haile T & Gemeda N (1979) Kala-azar in Ethiopia: survey of the south west Ethiopia, the leishmanin skin test and epidemiological studies. *Annals of Tropical Medicine and Parasitology* **70**, 417–431.

Gebre-Michael T & Lane RP (1996) The roles of Phlebotomus martini and *P. celiae* (Diptera: Phlebotominae) a s vectors of visceral leishmaniasis in the Aba Roba focus, southern Ethiopia. *Medical and Veterinary Entomology* **10**, 53–62.

Gebre-Michael T, Balkew M, Berhe N, Hailu A & Mekonnen Y (2010) Further studies on the phlebotomine sandflies of the kala-azar endemic lowlands of HumeraMetema (north-west Ethiopia) with observations on their natural blood meal sources. *Parasites & Vectors* **3**, 1–7.

Hailu A, Balkew M, Berhe N, Meredith SEO & Gemetchu T (1995) Is Phlebotomus (Larroussius) orientalisa vector of visceral leishmaniasis in south-west Ethiopia? *Acta Tropica* **60**, 15–20.

Hassan MM, Osman OF, El Raba FM *et al.* (2009) Role of domestic animals as a reservoir of *L. donovani* in Eastern Sudan. *Parasite and Vectors* **2**. doi:310.1186/1756-3305-2-26.

Hoogstraal H & Heyeneman D (1969) Leishmaniasis in the sudan republic: 30. Final report. *The American Journal of Tropical Medicine and Hygiene* **18**, 1091–1210.

Horst R, Collin SM, Ritmeijer K *et al.* (2008) Concordant HIV infection and visceral leishmaniasis in Ethiopia: the influence of antiretroviral treatment and other factors on outcome. *Clinical Infectious Diseases* **46**, 1702–1709.

Khanal B, Picado A, Bhattaria NM *et al.* (2010) Spatial analysis specially goats may play in role in the distribution of *L. donovani* exposure in human and domestic animals in recent kala azar focus in Nepal. *Parasitology* **137**, 1597–1603.

Kolaczinski JH, Reithinger R, Worku DT *et al.* (2008) Risk factors of visceral leishmaniasis in East Africa: a case-control study in Pokot territory of Kenya and Uganda. *International Journal of Epidemiology* **37**, 344–352.

Mengesha B & Abuhay M (1978) Kala azar among labour migrants in Metema and Humera regions of Ethiopia. *Tropical and Geographical Medicine* **30**, 199–206.

Mohebali M, Eddrisian GH, Nadim A *et al.* (2006) Application of Direct Agglutination Test (DAT) for the diagnosis and seroepidemiological studies of visceral leishmaniasis in Iran. *Iranian Journal of Parasitology* **1**, 15–25.

Mukhtar MM, Sharif AH, El Safi SH *et al.* (2000) Detection of antibodies of *Leishmania donovani* in animals in Kala azar endemic regions in eastern Sudan: preliminary report. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 33–36.

Ramos-Vara JA, Ortiz-Santiago B, Segalès J & Dunstan RW (1996) Cutaneous leishmaniasis in two horses. *Veterinary Pathology* **33**, 731–734.

Reithinger R, Brooker S & Kolaczinski J (2007) Visceral leishmaniasis in east Africa- current status. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 1169–1170.

Sixl W, Sebek F, Reinthaler F & Mascher F (1987) Investigations of wild animals as Leishmania reservoir in south Sudan. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology* **31**, 483–485.

Sollano–Gallego L, Roderiquez–Corties A, Inista L *et al.* (2000) Cross-sectional serosurvey of feline leishmaniasis in ecoregions around the north western Mediterranean. *American Journal of Tropical Medicine and Hygiene* **78**: 676–680.

Soulsby EJL (1982). *Helmenths, Arthropods and Protozoa of Domesticated Animals*, 7th edn. Bailliere Tindall, Philadelphia, PA, USA.

Sundar S & Rai R (2002) Laboratory diagnosis of visceral leishmaniasis. *Clinical and Diagnostic Laboratory Immunology* **9**, 951–958.

Villalobos EM, Caravalho PR, Lard MCC *et al.* (2010) Response of healthy equines with antileishmanial antibodies chagasi in an endemic area of leishmaniasis. *Middle East Journal of Scientific Research* **5**, 520–534.

Werneck GL, Costa CH, Walker AM *et al.* (2007) Multilevel modelling of the incidence of visceral leishmaniasis in Teresina, Brazil. *Epidemiology and Infection* **135**, 195–201.

WHO. (1991) *Report: Information on the Epidemiology and Control of Leishmaniasis by Country or Territory*. World health organization, Geneva. WHO/Leish/91-30.

Williams AO, Mutinga J & Rogers M (1991) Leishmaniasis in domestic goats in Kenya. *Molecular and Cellular Probes* **5**, 319–325.

Zijilstra EL, Ali MS, Hassan E et al. (1995) Endemic kala azar in eastern Sudan: PKDL. *The American Journal of Tropical Medicine and Hygiene* **52**, 299–305.

Corresponding Author Ambaye Kenubih, Department of Veterinary Microbiology, Immunology and Public Health, Faculty of Veterinary Medicine, Addis Ababa University, Debre Ziet, Ethiopia. E-mail: ambayken@yahoo.com